Angew. Botanik **61**, 25—37 (1987)

ISSN 006-1759/ASTM-Coden: ANBTAJ

Department of Botany, The University of Sheffield, UK

In Support of Frank's Organic Nitrogen Theory

By
D. J. Read

Summary

Frank's hypothesis that mycorrhizal infection could provide plants with access to organic nitrogen sources has been tested by growing ericaceous and ectomycorrhizal host plants in the infected and uninfected conditions with pure protein as sole nitrogen source. In both ericoid and ectomycorrhizal plants, infection leads to assimilation of protein N, and, as a result, to enhancement of plant yield and tissue nitrogen concentration. It is proposed that the infected plants, the roots of which are normally concentrated in the decomposition horizons of soil, would have direct access to protein N as it is released from the soil microflora and fauna, and that, as Frank suggested, mycorrhizal infection will thus play a key role in the nitrogen nutrition of the host plants. The implication of these observations for nitrogen and carbon cycling in forest ecosystems is discussed.

Introduction

After providing the first description of the ectomycorrhizal root in 1885, Frank became interested in the pattern of distribution of infected roots within soil. He noted (Frank, 1894) that in forest soils these roots were formed most prolifically in the organic horizons, an observation that has been substantiated by numerous workers since Frank's time. Frank logically deduced that mycorrhizal roots may be located in this part of the soil profile because they are adapted to exploit the particular resources accumulating in this region. Since nitrogen is quantitatively the most important plant nutrient in the organic matter Frank proposed, in what has become known as his 'organic nitrogen theory', that infection might provide access to the nitrogenous reserves contained in this material. While it has been demonstrated since Frank's time that ectomycorrhizal fungi have the ability to absorb small N containing molecules such as amino-acids, and transfer then to the plant (Melin, 1953), the possibility that they could provide access to polymeric forms of N has received relatively little attention.

Read (1985) noted that the accumulation of organic matter, which occurs along latitudinal or altitudinal gradients of increasing environmental stress, was associated with changes of mycorrhizal type. In the warmer climatic zones, rapid decomposition gives rise to soil of low organic content in which plants with vesicular-arbuscular (VA) mycorrhizas predominate. As seasonality becomes more prominent, organic matter begins to accumulate, first at the surface, and eventually, in more extreme parts of the gradient to greater depth, forming peat. In the soils with surface accumulation of organic

matter, plants with ectomycorrhizas predominate, while in the peaty soils these become replaced by ericaceous plants with their characteristic 'ericoid' mycorrhizas. Stribley and Read (1980) demonstrated that ericoid mycorrhizal infection enhanced the ability of *Vaccinium* to utilize amino-acids, but, if Frank's hypothesis was correct, it would be predicted that the transition from VA to ecto- or ericoid mycorrhizas arises through selection of those mycorrhizal types which are progressively more able to utilize polymeric organic N resources. Experimental evidence to support this hypoth
been produced and is summarized in this paper. More detailed description of these and related experiments can be found in Bajwa et al. (1985) and Abuzinadah and Read (1986).

Utilization of Organic N by Plants with Ericoid Mycorrhizas

The ability of mycorrhizal (M) and non-mycorrhizal (NM) plants of *Vaccinium macrocarpon* to utilize the protein Bovine Serum Albumen (BSA) was examined. BSA provides a 99 % pure protein containing 16 % N and has a molecular weight of 67,000. Strips of Whatman No. 1 paper were thoroughly washed, sterilized and laid over glass frames in aseptically assembled transparent plastic plant containers, so that the ends of the paper strips were immersed in the culture medium. The basal medium was that of Rorison ($\frac{1}{4}$ strength) from which all mineral N sources were excluded. Glucose (0.5 g l^{-1}) and active charcoal (1 g l^{-1}) were added to the medium as recommended by Duclos and Fortin (1983) after preliminary trials showed that they provided significant improvements of infection intensity in inoculated containers. The medium was poured over the paper and, at the same time, half of the containers were inoculated with a mycelial macerate of the ericoid mycorrhizal fungus *Hymenoscyphus ericae* (Read) Korf. & Kernan. After three days of incubation twelve aseptically germinated week old seedlings of *V. macrocarpon* were transferred to the paper platform in each container, and the containers were incubated for a further two weeks in growth chambers in conditions described above. At this stage examination of randomly selected root systems revealed consistently high infection levels, normal root growth, and balanced production of external mycelium in the inoculated containers. Protein was now added to half of the M and NM containers as 20 ml of a 0.06 % solution of BSA, giving 2 mg of N per container. After 30 d 24 plants (two containers) from the M and NM sets, in both BSA and 'no nitrogen' treatments, were harvested, oven-dried, weighed and digested in an H_2SO_4-Na_2SO_4-Se solution. Nitrogen contents of the digests were assayed by a modified Nessler procedure. Nitrogen contents of randomly selected seeds of *V. macrocarpon* were also determined so that starting N contents and gains from added protein could be discriminated.

Mycorrhizal plants grown on BSA had significantly higher shoot and total dry weights than the NM plants, the yields of which were similar to those found in M and NM treatments not provided with nitrogen (Table 1a, Plate 1a). This suggests that uninfected plants are unable to utilize the protein as an N source. Analysis of shoot and root nitrogen status confirms that infection leads to highly significant increases of shoot and total N content compared with the other treatments (Table 1b), and that no significant uptake of N occurred in the NM plants grown on BSA.

Table 1a: Dry weight yields (mg) of shoots, roots and whole plants of mycorrhizal and non-mycorrhizal *Vaccinium macrocarpon* after growth for 30 days on protein (BSA) as sole nitrogen source or without nitrogen.

Status	Treatment					
	BSA			No nitrogen		
	Root	Shoot	Whole Plant	Root	Shoot	Whole Plant
Mycorrhizal	1.40±2.0NS	4.86±1.6***	6.26±2.5***	1.21±2.2NS	4.02±1.2NS	5.25±1.4NS
Non-mycorrhizal	1.61±1.1	4.07±1.5	5.68±1.3	1.53±1.7	4.09±1.6	5.62±2.3

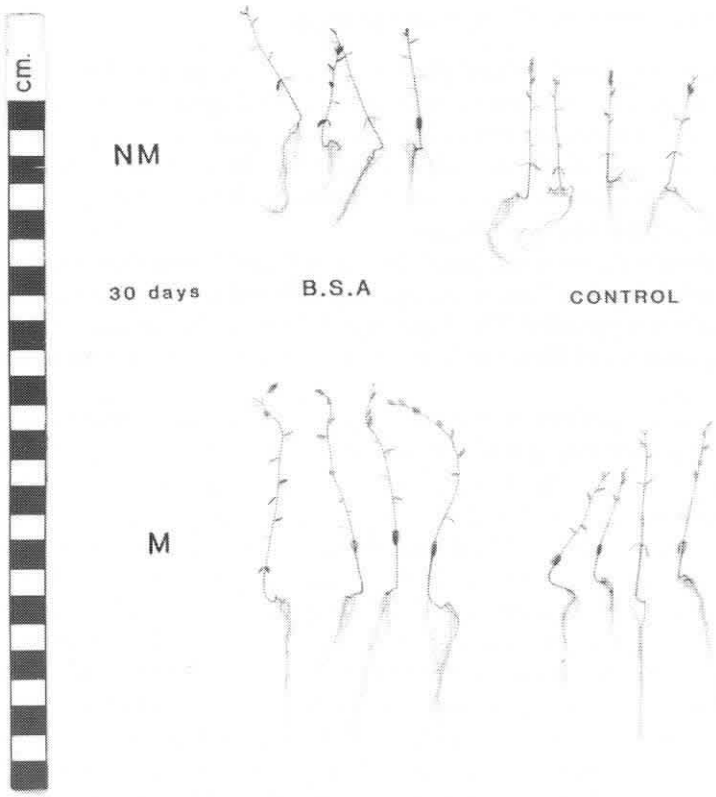

Plate 1a: Representative plants from mycorrhizal (M) and non-mycorrhizal (NM) treatments after 30 d growth with protein (BSA) and without nitrogen (control).

Table 1b: Nitrogen contents (μg) of shoots, root and whole plants of the same plants of *Vaccinium macrocarpon.*

Mycorrhizal	27 ± 5NS	95 ± 11***	121 ± 14***	15 ± 6NS	40 ± 7NS	56 ± 4NS
Non-mycorrhizal	23 ± 5	46 ± 6	70 ± 12	19 ± 3	39 ± 5	59 ± 3

*** Indicate significant difference between mycorrhizal and non-mycorrhizal plants within treatments at $P < 0.001$.

Root nitrogen levels of M plants were no higher than those of the NM treatment with BSA, indicating that nitrogen assimilated by the fungus passes rapidly to the shoots.

The total N content per seed of *V. macrocarpon* is 69 μg. Measurement of change of N levels at the end of the experiment shows that M plants grown with BSA gain 52 μg N per plant, while NM gain only 1 μg and the M and NM plants which received no exogenous N supply lost 13 and 10 μg N respectively, presumably as a result of leakage from their tissues.

In separate experiments it was shown that *H. ericae* had the ability to use number of peptides and proteins as sole sources of nitrogen when growing in pure culture.

Utilization of Organic N by Ectomycorrhizal Plants

The ability of ectomycorrhizal plants of *Pinus contorta* to utilize protein as a sole source of nitrogen was examined using an experimental approach similar to that adopted in the ericoid mycorrhizal system. In this case mycorrhizas were synthesized using the fungus *Paxillus involutus* in 250 ml Erlenmeyer flasks. Each flask contained 20 g sterilized perlite moistened with 50 ml of modified Melin-Norkrans solution (MMN) containing glucose but lacking nitrogen.

Two N treatments were employed. In one, mineral N was supplied in the form of ammonium sulphate, each flask receiving 5 mg of N before being autoclaved. In the second, protein N was provided. The flasks were autoclaved before receiving 5 mg of N in the form of a solution of BSA which had been passed through a millipore filter of 0.2 μ pore size.

Three discs of mycelium of *P. involutus* were added to the perlite in each flask and when mycelial growth had spread through the perlite, three seedlings were transferred aseptically to the flasks which were incubated in a controlled environment growth chamber (20 °C day — 15 °C night). Three flasks of each of the nitrogen treatments were harvested at 60, 90 and 120 d after planting. Levels of mycorrhizal infection, dry weights and nitrogen contents were determined at each harvest. Plants were divided into roots and shoots which were oven-dried, weighed and digested before their N contents were analysed as described for the ericoid system. Data from plant dry weight and nitrogen measurements obtained at the final harvests, were subjected to a two way analysis of variance to determine the overall significance of the treatment effects and their interactions. Differences between M and NM plants within the individual nitrogen treatments at every harvest were analysed using 't' tests. The proportion of the plant nitrogen content at each harvest which was derived from NH_4 or protein was calculated. The mean N content per seed was 200 μg and per flask (3 plants) 600 μg, which constitutes 11 % of the total N content of 5600 μg in each flask after commencement of the

Table 2: The significance of treatment effects and interactions as revealed by two-way ANOVAR of dry weight and nitrogen content of plants grown with *Paxillus involutus*

Treatment of Interaction		
Mycorrhizal Infection	Wt	ns
	N Content	P 0.01
Nitrogen Source	Wt	ns
	N Content	P 0.001
Mycorrhiza × Nitrogen Interaction	Wt	$P < 0.001$
	N Content	P 0.001

treatments. Thus any increase of plant nitrogen content above 600 μg per flask must represent a gain from the added N. In addition to this easily quantifiable value which is expressed in both weight and percentage terms, a considerable but unknown proportion of the added N must remain in the external mycelium.

The results of the ANOVAR test (Table 2) show that while there was no significant relationship between mycorrhizal infection or nitrogen source and plant weight, the relationships between both of these treatments and plant N content were significant. The mycorrhiza and nitrogen interaction was also significant in the case of both weight and N content.

Dry weight yields of mycorrhizal plants provided with protein N were significantly higher than those of non-mycorrhizal plants at Harvests 1 and 3 (Fig. 1). The appearance of the two categories of plants was strikingly different by Harvest 3 (Plate 1b). Though they were also larger at H_2 the differences were not significant. The reverse situation was obtained in the ammonium treatment where, at Harvests 2 and 3, the dry weight yields of NM plants were significantly larger than those of M plants. This reversal explains the failure to detect a significant relationship between infection and plant weight in the ANOVAR after the final harvest (Table 2). No differences between N contents of M and NM plants could be detected at the first harvest in either of the protein or ammonium treatments (Fig. 2). By the second harvest, however, N contents of M plants in the protein treatments were significantly higher than in the NM plants and it was noticeable that the latter showed no increase of N content at either of the harvests after Harvest 1. The M plants, in contrast, showed a steady increase of N with time. Nitrogen contents of NM plants grown on ammonium were significantly higher at Harvests 2 and 3 than were those of the M plants.

When the nitrogen budgets are calculated on a flask basis (Table 3) it is seen not only that the NM plants completely fail to utilize protein but also that their nitrogen contents decline relative to the 600 μg originally present in the three seeds added to the flasks. This loss is attributed to leakage of N from the starved seedlings. Increases of N equivalent to 10 % and 29 % respectively were, however, observed at H_2 and H_3 in the mycorrhizal plants. The increase at H_3 is higher than that obtained in M plants grown on ammonium at this harvest. In contrast to the situation seen in the protein treatment NM plants readily utilized ammonium. The difference in patterns of accumulation of N

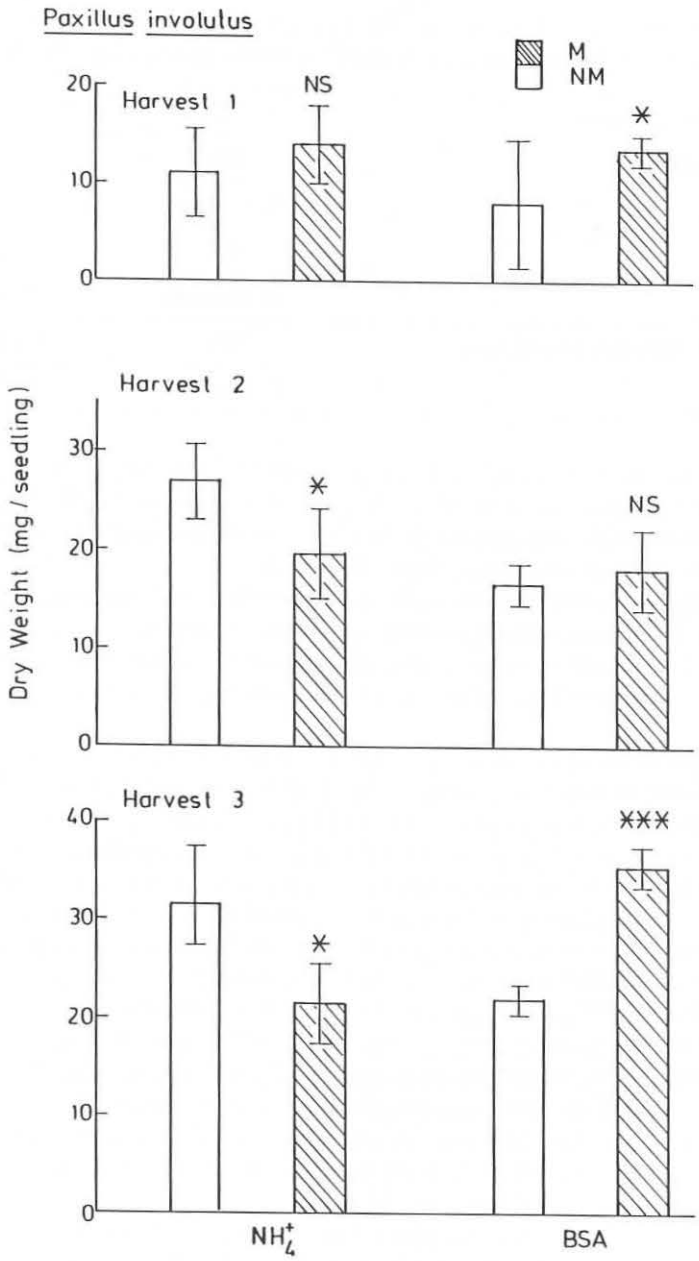

Fig. 1: Mean dry weight yields of *Pinus contorta* at three harvests after growth in the mycorrhizal condition with *Paxillus involutus* (M), or in the non-mycorrhizal condition (NM), with ammonium (NH_4+) or protein (BSA) as nitrogen source. *** indicates significant difference between M and NM plants at $P < 0.001$, ** at $P < 0.05$, * at $P < 0.01$. NS indicates no significant difference.

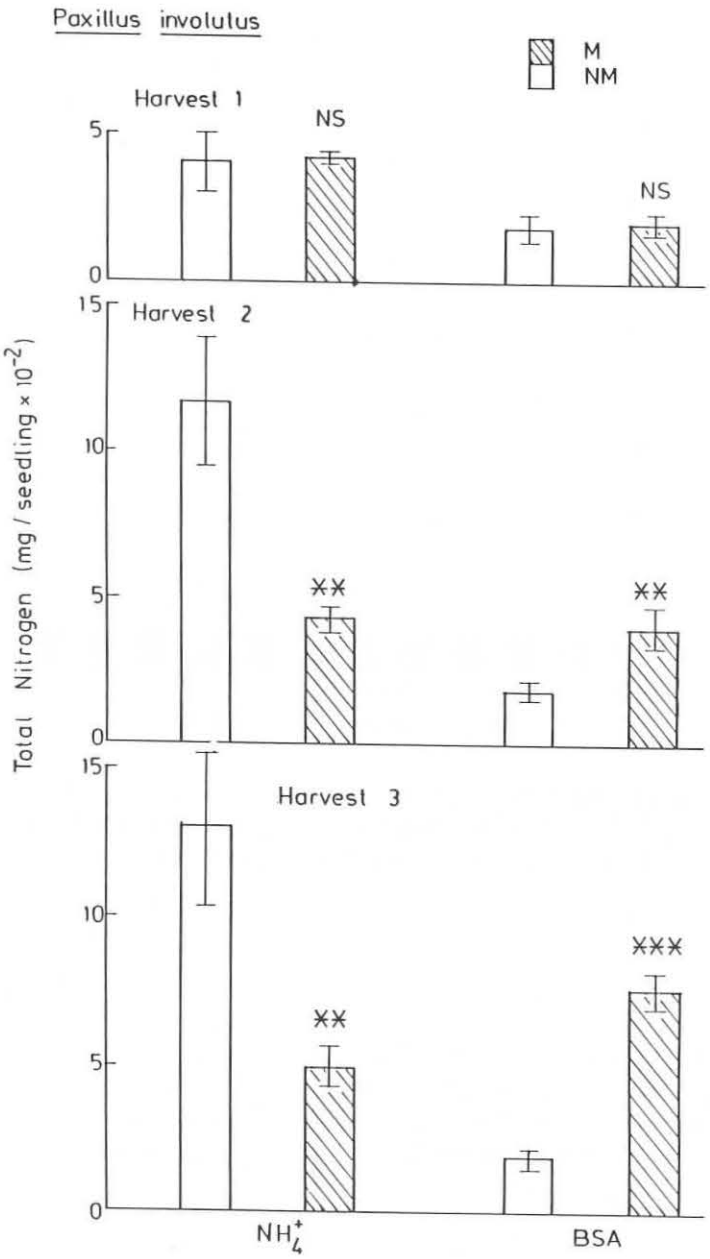

Fig. 2: As Fig. 1 but showing nitrogen contents of the plants at the three harvests.

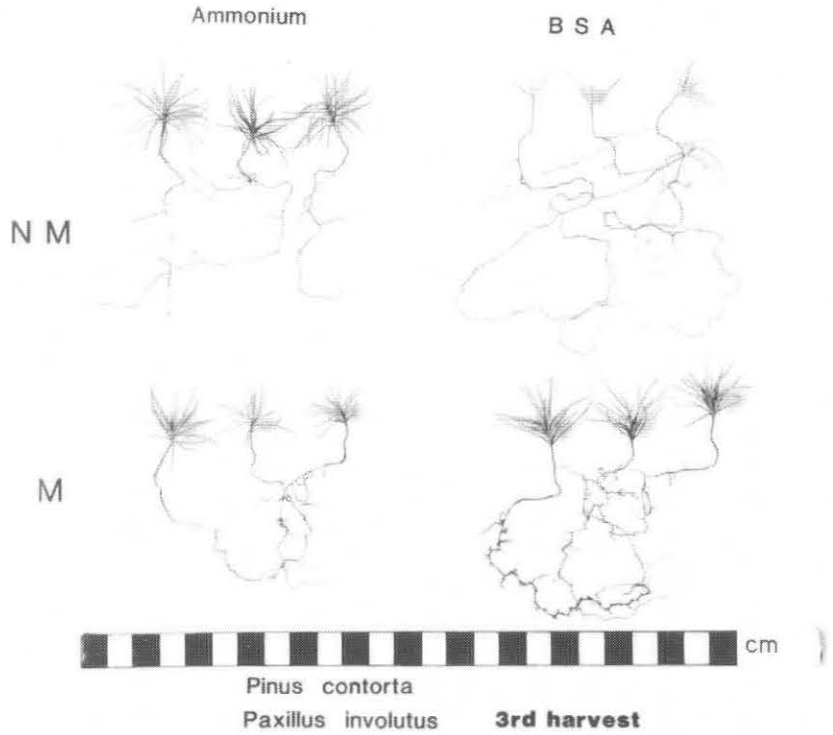

Plate 1b: Representative plants of *Pinus contorta* after growth for 120 d in the mycorrhizal (M) condition with *Paxillus involutus,* or in the non-mycorrhizal (NM) condition, with ammonium or protein (BSA) as sole nitrogen sources.

Table 3: Mean nitrogen contents (μg/flask) in plants infected with *P. involutus* and in non mycorrhizal plants at each of three harvest (H1, H2, H3) after growth on a mineral (NH_4+) or protein (BSA) nitrogen. Figures in brackets indicate the percentage of the plant nitrogen content which is derived from the added mineral or protein N.

	Treatment					
	NH_4+			BSA		
	H1	H2	H3	H4	H5	H6
Mycorrhizal (*P. involutus*)	1251 (11)	1287 (12)	1473 (15)	585	1194 (10)	2262 (29)
Non mycorrhizal	1185 (10)	3483 (51)	3660 (54)	552	548	566

in M and NM plants grown on mineral nitrogen probably arises because the mycorrhizal fungus competes with the plants as a potential sink for N.

Discussion

There has been much discussion of the organic nitrogen theory since it was proposed by Frank in 1894, but the hypothesis that mycorrhizas might provide direct access to the nitrogen of the organic matter in soil has not been widely tested in experiments using infected and uninfected plants. In those experiments that have been carried out, emphasis has been placed upon the ability of mycorrhizal plants to obtain nitrogen from humified material. Using this approach, Lundberg (1970) could find little evidence for the transfer of nitrogen from ^{15}N labelled humus to ectomycorrhizal plants. Stribley and Read (1974) provided indirect evidence for the use organic N by plants with ericoid infection, but later (1980) showed that the ericoid endophyte growing in pure culture had no access to the nitrogen contained in fulvic or humic acids. At first sight such results might suggest that Frank's hypothesis was invalid, and most recent reviews of the role of mycorrhizas in plant nitrogen nutrition have come to the conclusion, either that their primary role is enhancement of ammonium uptake (Alexander, 1983) or that raised N concentrations in infected plants arise only as a secondary consequence of their improved access to phosphate (Rousseau and Reid, 1985). However, the results presented here suggest that to assess Frank's view in terms of utilization only of fully humified or fully mineralized nitrogen is too simplistic. In acid forest soils both of these forms of nitrogen represent the end points of separate pathways each deriving from protein which is the primary substrate released into the environment as the soil biota turns over. Seen in this light, access to protein, appears to be a factor of key importance, while the ammonium ions arising from mineralization processes, and the humified nitrogen arising from protein-precipitation reactions, are both end products which may be of lesser consequence for nutrition of the ectomycorrhizal plant.

Earlier studies of the utilization of protein by ectomycorrhizal fungi showed that some species could readily obtain nitrogen from such sources (Melin, 1925; Mikola, 1948). Others, in contrast, indicated only a weak ability to assimilate protein nitrogen (Mosca and Fontana, 1975). It has recently been revealed that the ericoid mycorrhizal endophyte, *H. ericae,* has a clear ability to use proteins of several different types (Spinner and Haselwandter, 1985; Read and Bajwa, 1985). However, the critical feature revealed in the present results, and discussed in more detail elsewhere (Bajwa et al., 1985; Abuzinadah and Read, 1986) is that the nitrogen assimilated from the protein is not immobilized in the vegetative mycelium of the fungus, but transferred rapidly to the host plant. This is the pattern of events envisaged by Frank, and the current observations are thus thought to go some way towards providing a vindication of his view.

Detailed analysis of the distribution of fine roots of both ericoid (Bannister, 1966; Chapman, 1970; Gimingham, 1972; Persson, 1980) and ectomycorrhizal (Harley, 1940; Wehrlich and Lyr, 1957; Mikola and Laiho, 1962; Meyer, 1973; Harvey et al., 1976; Deans, 1979; Alexander and Fairley, 1983) plants have shown that the majority of mycorrhizal roots are situated not in the fully humified 'H' layers but in the decomposition or 'F' horizons which lie between the litter 'L' and

humus horizons. It as been shown (Harley, 1940) that in beech, mother roots lie horizontally over the surface of the humus layer, producing mycorrhizal laterals that grow upwards into the litter. Within the 'F' horizon extensive wefts of fungal mycelium, some at least being formed by the mycorrhizal fungi, are testament to the high concentrations of microbial biomass occurring in the vicinity of mycorrhizal roots. Both the pattern of distribution of the roots and the visible evidence of extensive microbial growth thus suggest that the site of nitrogen assimilation is more likely to be the F than the H horizon. In this situation the unhumified protein nitrogen being released from the rapidly turning over soil micro-flora and fauna is likely to be the most important source of the element. Concentrations of N in soil micro-organisms are particularly high, values in excess of 4 % being reported (Ausmus et al., 1976). For this reason the turnover of the microbial population has been recognized to be a key factor in the mobilization of macro-elements in organic soils (Heal, 1979). Since the mycorrhizal fungi are intimately connected with horizons in which this turnover is taking place most extensively, their proteolytic capability is likely to be of vital importance as a component of the nitrogen cycle. They represent a major sink for protein, the nitrogen of which, as the present experiments demonstrate, can be rapidly transferred to the autotroph rather than being re-immobilized. Thus, in stable forest ecosystems dominated by ectomycorrhizal plants, the mycorrhizal fungi not only provide access to an otherwise unavailable source of nitrogen but also provide a much tighter cycling of the element than hitherto has been proposed. By assimilating protein, including that from their own mycelia, and returning it directly to the tree, these fungi have the capacity to reduce the importance of the longer and more inefficient mineralization pathways in which the intervention of a separate decomposer population is involved. They will also gain significant quantities of carbon and thus reduce the drain on host assimilates.

In soil, competition for protein nitrogen between saprotrophic and mycotrophic fungi would be expected but the symbiotic organisms have the advantage that they are attached to the continuous supply of free carbon which is required for the synthesis of proteolytic enzymes. Selective exploitation of organic N resources by this group of micro-organisms would help to explain the observation of Gadgil and Gadgil (1971, 1975), that exclusion of mycorrhizal roots from pine litter led to a dramatic increase in its rate of decomposition. Studies of Park (1976) have shown that cellulose decomposition is markedly inhibited if N supply to the saprotrophic population is restricted. The ability of ectomycorrhizal fungi to compete successfully for nitrogen could lead to N starvation and consequently to reduce cellulolytic activity amongst decomposers. The magnitude of the so called 'Gadgil effect' in any forest environment may therefore depend upon the availability of nitrogen, the effect being greatest in those circumstances where extensive ectomycorrhizal mycelial systems are effectively scavenging for limited N supplies.

While, in the laboratory experiments, protein is continuously available to the mycorrhizal fungi, in the field it is likely that there will be a seasonal cycle of release and immobilization. In spruce forest, for example, a rapid increase in microbial biomass in spring and summer, has been shown to produce a net immobilization of N (Flanagan and Van Cleve, 1977), there being release of N in late summer and autumn which was calculated to be equivalent to 0.24 g N/sq m. It is interesting that this release, coincides with a peak period of production of mycorrhizal roots in a number of coniferous

species (Vogt et al., 1980; Alexander and Fairley, 1983). The mycorrhizas are therefore well placed in both temporal and spatial terms to intercept organic N compounds as their mobilization proceeds.

In his discussion of the possible role of mycorrhizas in plant nutrition, Frank (1894) pointed out that as an inevitable consequence of absorption of organic N, carbon supplies to the fungus and host would be supplemented. There has been much subsequent debate about the role of the mycorrhizal fungus in the carbon nutrition of the host, some workers concluding that the fungus could assimilate carbon derived from soil carbohydrates and supply them to the autotroph (McDougal and Dufrenoy, 1944, 1946; Young, 1947) while others have argued against the possibility that such transfer could represent a significant source of carbon for the plant (Harley, 1952; Lewis and Harley, 1965). Recent research demonstrating that some mycorrhizal fungi have the ability to release carbon from lignin, holocellulose and ligno-cellulose (Trojanowski et al., 1984), together with the present observation of extensive proteolytic activity, lends further support to the view that the carbon requirements of the heterotrophs can be supplemented from sources available in soil, and highlight the need for more information on transfer of carbon from fungus to host. The results reported in this paper already provide substantial support for Frank's view that mycorrhizas were intimately associated with the mobilization of organic resources of the soil. Further studies in the laboratory perhaps using ^{14}C and ^{15}N labelled tracers, and in the soil, to determine the pattern of protein turnover, are required before the final vindication of Frank's ideas can be obtained.

Zusammenfassung

Aufnahme von organischem Stickstoff durch Mykorrhizen: Unterstützung von Franks Theorie

Franks Hypothese, daß die Mykorrhizainfektion den Pflanzen Zugang zu organischen Stickstoffquellen verschaffen kann, wurde dadurch geprüft, daß Erikaceen- und Ektomykorrhiza-Wirtspflanzen, jeweils infiziert und nicht infiziert, mit reinem Protein als einziger Stickstoffquelle kultiviert wurden. In beiden Fällen führte die Infektion zur Assimilation von Protein-Stickstoff und, als Folge davon, zu einer Zunahme von Pflanzenertrag und Stickstoffgehalt des Pflanzengewebes. Es wird vermutet, daß die Wurzeln der infizierten Pflanzen Zugang zu dem Protein-Stickstoff haben, den Mikroflora und -fauna in ihrer direkten Umgebung freisetzen und daß, wie Frank vorschlug, die Mykorrhizainfektion auf diese Weise eine Schlüsselrolle bei der Stickstoffversorgung der Wirtspflanze spielt. Die Bedeutung dieser Beobachtung für den Stickstoff- und Kohlenstoffkreislauf in Waldökosystemen wird diskutiert.

References

Abuzinadah, R. A. and Read, D. J., 1986: The role of proteins in the nitrogen nutrition of ectomycorrhizal plants. II. Utilization of protein by mycorrhizal plants of *Pinus contorta.* New Phytologist (in press).

Alexander, I. J., 1983: Ectomycorrhizas in the nitrogen cycle. In: J. A. Lee, S. McNeil and I. H. Rorison (eds.), Nitrogen as an ecological factor, pp. 69—93. Blackwell, Oxford.

Alexander, I. J. and Fairley, R. I., 1983: Effects of N fertilization on populations of fine roots and mycorrhizas in spruce humus. Plant and Soil **71,** 49—53.

Ausmus, B. S., Edwards, N. T. and Witkamp, M., 1976: Microbial immobilization of carbon, nitrogen, phosphorus and potassium: implications for forest ecosystem processes. In: J. M. Anderson and A. Macfadyen (eds.), The role of terrestrial and aquatic organisms in decomposition processes, pp. 397—416. Blackwell, Oxford.

Bajwa, R. and Read, D. J., 1985: The Biology of Mycorrhiza in the Ericaceae. IX. Peptides as nitrogen sources for the ericoid endophyte and for mycorrhizal and non-mycorrhizal plants. New Phytologist (in press).

Bajwa, R., Abuarghub, S. and Read, D. J., 1985: The Biology of Mycorrhiza in the Ericaceae. X. The Utilization of proteins and the production of proteolytic enzymes by the mycorrhizal endophyte and by mycorrhizal plants. New Phytologist (in press).

Chapman, S. B., 1970: The nutrient content of the soil and root system of a dry heath ecosystem. J. Ecology **58,** 445—452.

Deans, J. D., 1979: Fluctuations of the soil environment and fine root growth in a young Sitka Spruce plantation. Plant and Soil **52,** 195—205.

Duclos, J. L. and Fortin, J. A., 1983: Effect of glucose and active charcoal on in vitro synthesis of ericoid mycorrhizas with *Vaccinium* spp. New Phytologist **94,** 95—102.

Flanagan, P. W. and Van Cleve, K., 1977: Microbial biomass, respiration and nutrient cycling in a black spruce taiga ecosystem. In: U. Lohm and T. Persson (eds.), Soil organisms as components of ecosystems. Ecological Bulletin **25,** 261—273. Stockholm.

Frank, A. B., 1894: Die Bedeutung der Mykorrhizapilze für die gemeine Kiefer. Forstwissenschaftliches Centralblatt **16,** 1852—1890.

Gadgil, R. L. and Gadgil, P. D., 1971: Mycorrhiza and litter decomposition. Nature **233,** 133.

Gadgil, R. L. and Gadgil, P. D., 1975: Suppression of litter decomposition by mycorrhizal roots of *Pinus radiata.* New Zealand J. Forest Science **5,** 33—41.

Gimingham, C. H., 1972: Ecology of Heathlands. Chapman & Hall, London.

Harley, J. L., 1940: A study of the root system of the beech in woodland soils with especial reference to mycorrhizal infection. J. Ecology **28,** 107—117.

Harley, J. L., 1952: Associations between micro-organisms and higher plants (Mycorrhiza). Ann. Rev. Microbiology **6,** 367—386.

Harvey, A. E., Larsen, M. J. and Jurgensen, M. F., 1976: Distribution of ectomycorrhizae in a mature Douglas fir/larch forest system in Western Montana. Forest Science **22,** 393—398.

Heal, O. W., 1979: Decomposition and nutrient release in even-aged plantations. In: E. D. Ford, D. C. Malcolm and J. Atterson (eds.), The ecology of even-aged forest plantations. Institute of Terrestrial Ecology. Cambridge, UK.

Lewis, D. H. and Harley, J. L., 1965: Carbohydrate physiology of mycorrhizal beech roots. III. Movement of sugars between host and fungus. New Phytologist **64,** 256—269.

Lundeberg, G., 1970: Utilization of various nitrogen sources, in particular bound soil nitrogen, by mycorrhizal fungi. Studia Forestalia Suecica **79,** 1—95.

MacDougal, D. T. and Dufrenoy, J., 1944: Mycorrhizal symbiosis in *Aplectrum, Corallorhiza* and *Pinus.* Plant Physiology **19,** 440—465.

MacDougal, D. T. and Dufrenoy, J., 1946: Criteria of nutrition relations of fungi and seed plants in mycorrhizae. Plant Physiology **21,** 1—10.

Melin, E., 1925: Untersuchungen über die Bedeutung der Baummykorrhize. G. Fischer, Jena.

Melin, E. and Nilsson, H., 1953: Transfer of labelled nitrogen from glutamic acid to pine seedlings through the mycelium of *Boletus variegatus* (S. W.) Fr. Nature **171,** 434.

Meyer, F. H., 1973: Distribution of mycorrhizae in native and man-made forests. In: G. C. Marks and T. T. Kozlowski (eds.), Ectomycorrhizae. Academic Press, New York.

Mikola, P., 1948: On the physiology and ecology of *Cenococcum graniforme.* Communicationes Instituti Forestalis Fenniae **36,** 7—104.

Mikola, P. and Laiho, O., 1962: Mycorrhizal relations in the raw humus layer of northern spruce forests. Communicationes Institutum Forestalis Fenniae **55,** 1—13.

Mosca, A. M. L. and Fontana, A., 1975: Sull utilizzazione dell azoto proteico da paste dell micelio di *Boletus luteus* L. Allionia **20,** 47—52.

Park, D., 1976: Carbon and nitrogen levels as factors influencing fungal decomposers. In: J. M. Anderson and A. Macfadyan (eds.), The role of Terrestrial and Aquatic Organisms in Decomposition Processes, pp. 41—59. Blackwell, Oxford.

Persson, H. A., 1983: The distribution and productivity of fine roots in boreal forests. Plant and Soil **71,** 87—101.

Read, D. J., 1985: The structure and function of the vegetative mycelium of mycorrhizal roots. In: D. H. Jennings and A. D. M. Rayner (eds.), The Ecology and Physiology of the Fungal Mycelium. Cambridge University Press, Cambridge.

Read, D. J. and Bajwa, R., 1985: Some nutritional aspects of the biology of ericaceous mycorrhizas. Proceedings Royal Society Edinburgh **85B,** 317—332.

Rousseau, J. and Reid, C. P. P., 1985: Interaction of nitrogen phosphorus and mycorrhizae inoculation on nutrient content and growth of *Pinus contorta.* In: R. Molina (ed.), Proceedings of the 6th North American Conference on Mycorrhizae. Forest Research Laboratory, Oregon State University, Corvallis, Oregon.

Spinner, S. and Haselwandter, K., 1985: Proteins as nitrogen sources for *Hymenoscyphus (= Pezizella) ericae.* In: R. Molina (ed.), Proceedings of the 6th North American Conference on Mycorrhizae. Forest Research Laboratory, Oregon State University, Corvallis, Oregon.

Stribley, D. P. and Read, D. J., 1974: The biology of mycorrhiza in the Ericaceae. IV. The effect of mycorrhizal infection on uptake of ^{15}N from labelled soil by *Vaccinium macrocarpon* Ait. New Phytologist **73,** 1149—1155.

Stribley, D. P. and Read, D. J., 1980: The biology of mycorrhiza in the Ericaceae. VIII. The relationship between mycorrhizal infection and the capacity to utilize simple and complex organic nitrogen sources. New Phytologist **86,** 365—371.

Trojanowski, J., Haider, K. and Hutterman, A., 1984: Decomposition of ^{14}C-labelled lignin, holocellulose and lignocellulose by mycorrhizal fungi. Archiv Microbiol. **139,** 202—206.

Vogt, K. A., Grier, C. C., Meier, C. E. and Edmunds, R. L., 1982: Mycorrhizal role in net primary production and nutrient cycling in *Abies amabilis* (Dougl.) Forbes ecosystems in Western Washington. Ecology **63,** 370—380.

Werlich, I. and Lyr, H., 1957: Über die Mykorrhizaausbildung von Kiefer (*Pinus sylvestris* L.) und Buche (*Fagus sylvatica* L.) auf verschiedenen Standorten. Archiv Forstwiss. **6,** 1—23.

Young, H. E., 1947: Carbohydrate absorption by the roots of *Pinus taeda.* Queensland J. Agricult. Sci. **4,** 1—6.

Address of the author:
Dr. D. J. Read, Department of Botany, The University of Sheffield, S10 2TN, Sheffield, UK.